Research Paper

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Cell Proliferation and Collagen Synthesis Are Two Independent Events in Human Atherosclerotic Plaques

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Cell proliferation
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Arteries, human

Abstract
We have used a double-immunolabelling technique on human carotid atherosclerotic plaques to measure cell proliferation and type-I collagen gene expression, using antibodies to proliferating cell nuclear antigen (PCNA) and type-I procollagen protein, respectively. Although cell proliferative activity and type-I collagen gene expression can occur simultaneously in the same cell, this is a rare event, and the vast majority of collagen-producing cells do not show proliferative activity. These two processes also tend to occur in separate locations, although they can coexist in certain regions of the plaque. This disparate location of these two important modes of plaque growth suggests that cell proliferation and collagen gene expression may be under separate biological controls during the development and evolution of human atherosclerosis.

Introduction

Cell proliferation and extracellular matrix synthesis are assumed to be the main mechanisms of growth in atherosclerosis [1]. Since several growth factors which can stimulate smooth muscle cells to proliferate can also stimulate these cells to synthesize collagen [2–5], one might assume that these two processes occur in a simultaneous, coordinated fashion during atherosclerosis development. However, based on cell culture studies, the presence of a tight correlation between smooth muscle proliferation and collagen synthesis appears to be controversial. Some studies have indicated an inverse correlation between arterial smooth muscle collagen synthesis and the proliferative state [6, 7]. Other studies appear to demonstrate that proliferating smooth muscle cells have elevated collagen synthesis (on a per cell basis), regardless of whether cells were rendered quiescent by density arrest or by mitogen deprivation [8]. This is in concert with smooth muscle cell phenotypic modulation from a contractile state to a synthetic state [9, 10]. Yet to the best of our knowledge, no previous studies have tried to correlate these two processes of intimal growth in actual human atherosclerotic material.

Recently, we used an antibody to the proliferating cell nuclear antigen (PCNA), a cell cycle-specific protein [11, 12], and an anti-type-I procollagen antibody, the cytoplasmic precursor to mature type-I collagen [13], for separate studies of cell proliferation and collagen synthesis in human atherosclerosis. In the latter case, we found that type-I procollagen protein and mRNA were spatially correlated in human atherosclerotic plaques and essentially

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absent from normal arterial wall samples. We have therefore used type-I procollagen protein presence as an index of active type-I collagen synthesis, and have chosen to study this particular collagen gene since type-I collagen appears to be the most abundant collagen form in human atherosclerosis. An underlying assumption of this paper is that immunostainable procollagen denotes sites of active mature collagen synthesis. This assumption appears reasonable given the correlation we and others have found between procollagen immunocytochemistry and mRNA detected by in situ hybridization [13–15], and its presence during the phase of active wound healing in skin [16]. In the present study, we applied a double-immunolabeling technique to human atherosclerotic plaque material to reveal the spatial correlations between cell proliferation and collagen synthesis on the same tissue sections.

The following questions have been addressed. (1) Are arterial mesenchymal cells (smooth muscle-derived or endothelial) able to proliferate and produce collagen simultaneously? (2) If yes, do the majority of type-I collagen gene-expressing cells also display proliferative activity? (3) Are mesenchymal cell proliferation and collagen synthesis spatially correlated within the plaque, possibly implying common growth regulatory factors?

Materials and Methods

Tissue Preparation

We studied 12 human carotid artery plaques removed at the time of endarterectomy surgery. As control arterial tissue, portions of 11 human internal mammary arteries, not used for coronary bypass surgery, were obtained. These specimens were fixed overnight in methanol-Carnoy's fixative (methanol:chloroform:glacial acetic acid in a 60:30:10 volumne ratio), paraffin embedded and sectioned (section thickness was 5 μm). We received 1 plaque/patient, and placed representative portions (often the whole plaque, depending on its size) into 1 tissue block (i.e. 1 block/patient). From each block, several serial sections were generated for hematoxylin eosin staining as well as for the subsequent immunocytochemistry studies. When necessary, the specimens were decalcified in 2% *L*-ascorbic acid for 3 days, and this procedure did not appear to affect our immunostaining patterns. As positive tissue controls for PCNA and Ki-67 staining for cell proliferation, human tonsil (for both of them) and rat small intestine (for PCNA only) were used. As positive tissue controls for procollagen-I protein expression, human hypertrophic scar tissue and cultured rat lung fibroblasts were used. They gave the expected well-localized proliferative and type-I collagen synthetic patterns, respectively (prominent staining among the interstitial crypt epithelium and tonsil follicular centers for PCNA, similar pattern of Ki-67 staining of tonsil tissue, and cytoplasmic staining among dermal fibroblasts for type-I procollagen, data not shown). These studies were approved by the Human Subjects Review Committee at the University of Michigan (Ann Arbor, Mich., USA).

Immunocytochemistry

Sections from each sample were reacted with the following antibodies. Anti-PCNA (PC10) antibody was obtained from DAKO Corporation, (Carpenteria, Calif., USA). For independent confirmation of PCNA data, we used MIB-1 monoclonal antibody (AMAC, Inc., Westbrook, Me., USA), directed against cell cycle specific Ki-67 protein. Mouse anti-sheep procollagen-I amino-terminal monoclonal antibody SPI.D8 [17] was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, John Hopkins University School of Medicine (Baltimore, Md., USA) and the Department of Biological Sciences, University of Iowa (Iowa City, Iowa, USA), under contract N01-HD-6-2915 from NICHD. The other anti-procollagen antibody used on these tissues, as a kind of independent confirmation of SPI.D8 data, was a rat anti-human procollagen-I amino-terminal monoclonal antibody (MAB1912, Chemicon International, Inc., Temecula, Calif., USA). This antibody gave the same immunocytochemical patterns of these arterial tissues, as did SPI.D8 (data not shown). Single-label immunocytochemistry was done using our previously published protocols [13].

For double-label immunocytochemistry, sections were deparaffinized and, using 2% normal horse serum as diluent, the first primary antibody (PC10, dilution 1:1000) was applied overnight at 4°C. The secondary antibody incubation (biotinylated horse anti-mouse antibody, dilution 1:1,000, from Vector Laboratories, Burlingame, Calif., USA) was for 3 h at 4°C followed by avidin-biotin amplification (ABC kit from Vector Laboratories) for 30 min. Incubation with 0.1% 3'3' diaminobenzidine (Sigma Chemical Co., St. Louis, Mo., USA), H_2O_2 and nickel chloride at 37°C for 5–10 min produced a black reaction product. After the first immunostaining procedure, the sections were washed copiously in PBS and the second primary antibody (SPI.D8 at 1:10 dilution) was applied overnight at 4°C. The biotinylated secondary antibody to the second primary antibody (biotinylated horse anti-mouse antibody, dilution 1:500, from Vector Laboratories) was then applied for 3 h at 4°C, followed by another 30-min incubation in streptavidin-alkaline phosphatase (from Vector Laboratories, diluted at 1:1,000) and then developed with the alkaline phosphatase substrate (alkaline phosphatase substrate kit I, from Vector Laboratories) which produced a red reaction product. Methyl green counterstaining was used to visualize nuclei in the tissue sections. As controls for the immunostaining, reactions with normal mouse serum gave no specific staining of any structures (data not shown). Reactions to vimentin stained essentially every cell in the arterial tissue as expected (data not shown). Single labelling of representative serial sections separately for PCNA or type-I procollagen revealed that this double-immunolabelling protocol reliably reproduced the respective cell-staining patterns in the different plaque regions (data not shown).

Morphometric Analysis

To quantitatively study the distribution of PCNA- and procollagen-I-positive cells, tissue sections were studied by light microscopy at a 400 magnification using the Image I system of computer color image analysis (Universal Imaging Corporation, West Chester, Pa., USA). Contiguous, nonoverlapping microscopic fields (200 × 200 field size) were analyzed covering the whole specimen on the slide.

Each field was scored for: (1) the number of procollagen-positive cells; (2) the number of PCNA-positive cells, and (3) the number of double-labeled cells.

From this data set, we derived: (4) the number of fields containing only procollagen-positive cells; (5) the number of fields contain-

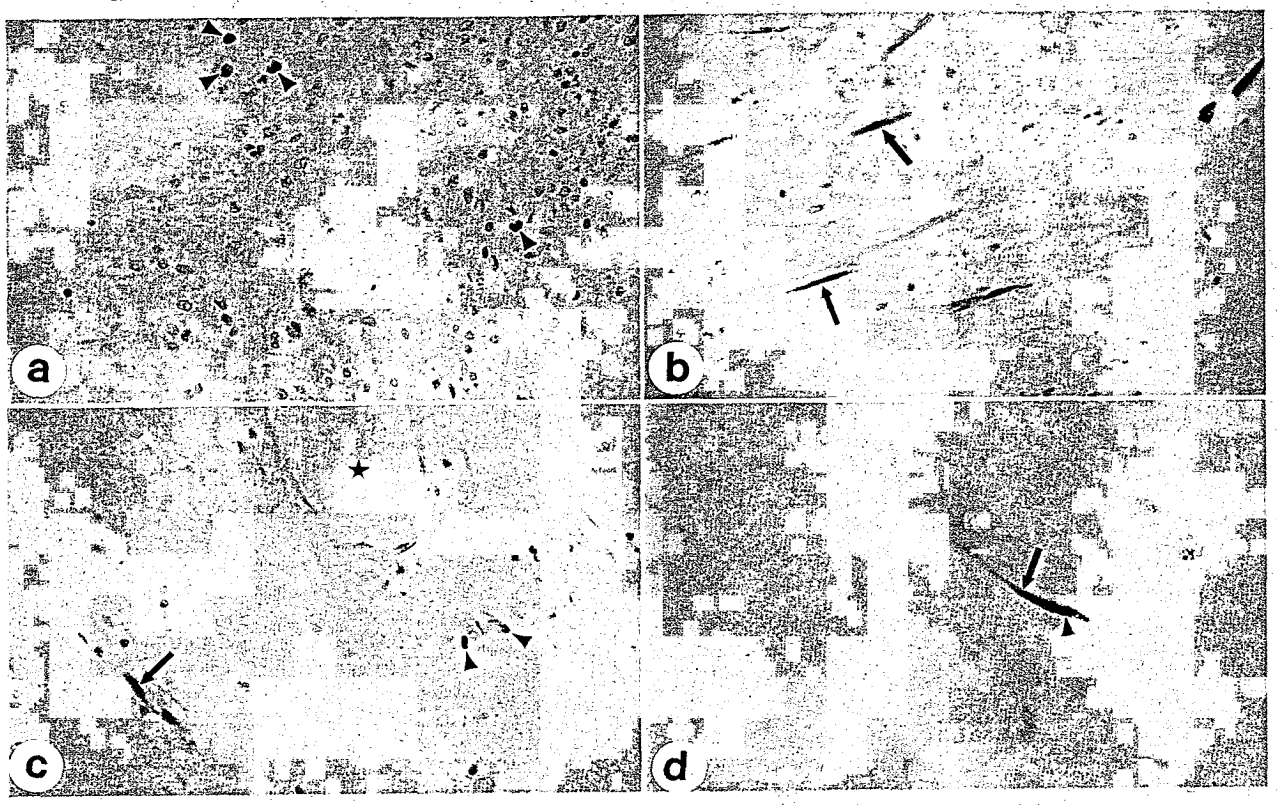

Fig. 1. Spatial patterns of cell proliferation and type-I collagen synthesis in human carotid plaque (according to double-label immunocytochemistry). Proliferating cells exhibit black PCNA-positive nuclei (product of the peroxidase reaction, arrowheads), collagen-I-producing cells exhibit red SPI.D8-positive cytoplasm (product of the alkaline phosphatase reaction, arrows). Light green counterstain shows PCNA-negative nuclei. **a** PCNA-positive cells in foam cell-rich region around necrotic core. × 326. **b** Procollagen-positive cells in the fibrous cap region. × 326. **c** PCNA-positive cells and procollagen-positive cells coexist in vascularized regions of the plaque. Star = Microvessel. × 326. **d** Double-labelled cell. × 815.

ing only PCNA-positive cells, and (6) the number of fields containing both PCNA-positive and procollagen-positive cells.

The data from all the specimens were pooled and Spearman's rank correlation coefficient was generated to study the correlation between the number of procollagen-positive and PCNA-positive cells in each field, using statistical package True Epistat (Epistat Services, Richardson, Tex., USA).

Results

The hematoxylin eosin stains of all internal mammary artery samples revealed a normal arterial morphology with minimal to absent intima (0–2 smooth muscle-like cells were generally present between the endothelium and the internal elastica). The carotid plaques were all advanced, complicated atherosclerotic plaques revealing necrotic cores, fibrous caps, frequent calcification, focal neovascularization, and occasional foci of intraplaque hemorrhage.

As for single antibody labelling, sections of internal mammary arteries revealed no reactivity to either proliferation marker (PCNA or Ki-67 antigen). The carotid plaques revealed a very low level of reactivity to either anti-PCNA or anti-Ki-67 antigen antibodies (0–1% of nuclei). With the type-I procollagen antibody (SP1.D8) we achieved the same staining patterns as with the double immunolabelling described below.

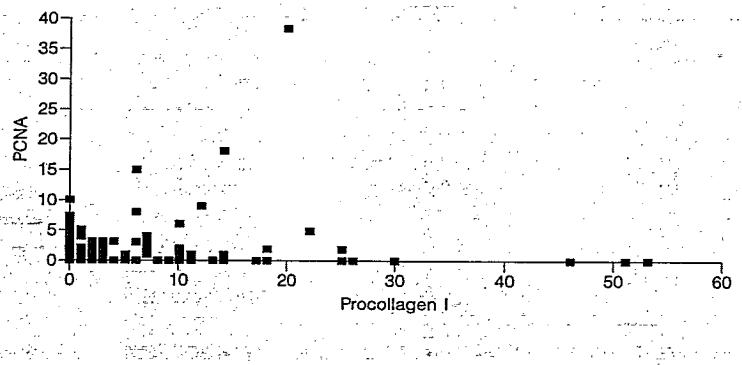

Fig. 2. The number of procollagen-I-positive cells vs. the number of PCNA-positive cells per microscopic field (455 fields analyzed). Note: there were only 132 fields positive for either procollagen or PCNA out of a total of 455 fields studied, putting the vast majority of fields at the origin.

Double immunolabelling of the internal mammary artery samples revealed neither PCNA nor type-I procollagen immunoreactivity in any of the samples, in agreement with previous studies [11, 13].

Double immunolabeling revealed the following classes of cells within the human carotid plaques (fig. 1): (1) 'quiescent' (showing neither PCNA nor procollagen-I staining); (2) PCNA-positive, procollagen-negative (fig. 1a); (3) PCNA-negative, procollagen-positive (fig. 1b), and (4) double positive (fig. 1d). The last category proves that in human atherosclerotic plaques, vascular cells can proliferate and synthesize collagen at the same time. However, this event is extremely rare. The percent of double-positive cells among procollagen-positive cells was only 0.68 % (5 of 734 cells). Therefore, although proliferation and collagen synthesis can occur in the same cells, the vast majority of collagen-producing cells do not show proliferative activity.

A graph, plotting the number of procollagen-positive cells per microscopic field vs. the number of PCNA-positive cells in each of the same microscopic fields does not show any visually obvious (positive or negative) correlation (fig. 2). However, Spearman's rank correlation analysis revealed a negative correlation between the numbers of procollagen- and PCNA-positive cells in each field ($r = -0.4197$, two-tailed $p < 0.000001$). Therefore, the more proliferating cells in a field, the less collagen synthesis can be expected. We recognize that the pooling of all of the microscopic field data from all the carotid plaques studied assumes that each field measurement is an independent event, an assumption which may not be valid. However, given that only 27.3% of all microscopic fields was positive for either PCNA or procollagen, we pooled these data in order to obtain enough data points for a meaningful correlation analysis.

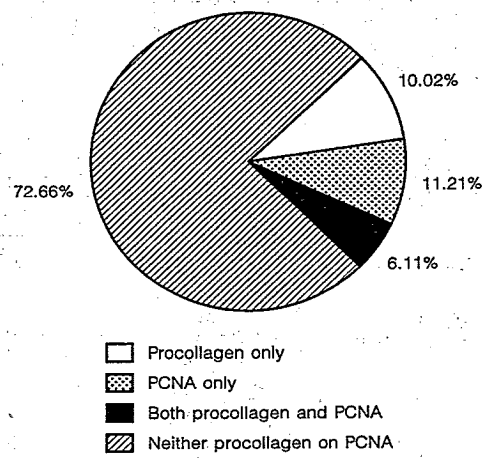

Fig. 3. Percent of microscopic fields containing at least one PCNA-positive and/or procollagen-I-positive cell (455 fields analyzed).

The above analyses were applied to the whole plaque irrelevant to particular plaque regions. However, we found that at least four kinds of regions can be highlighted within the plaque (fig. 3): (1) containing only procollagen-

positive cells (10.02%, fig. 1b); (2) containing only PCNA-positive cells (11.21%, fig. 1a); (3) containing both of them (6.11%, fig. 1d), and (4) containing neither procollagen nor PCNA positivity (72.67%). Qualitatively, most 'isolated collagen synthesis' occurred in the fibrous cap regions (fig. 1b), whereas most 'isolated proliferation' occurred in the foam cell-rich region around the necrotic cores (fig. 1a). Finally, collagen synthesis and cell proliferation most often coexisted in vascularized regions of the plaque (fig. 1c).

Discussion

This study of advanced atherosclerotic plaques from human carotid arteries again shows that ongoing plaque growth is focal and not a diffuse process occurring throughout the entire intima. As the data of fig. 3 indicate, 72.6% of the plaque shows no ongoing growth-related activity, either in terms of cell proliferation or type-I collagen gene expression (here used as a rough index of ongoing extracellular matrix production). The controls of these growth activities, and why only certain plaque regions appear to display ongoing growth, are not clear.

At the cellular level we have shown that proliferation and collagen synthesis can occur in the same cell. However, the vast majority of collagen-producing cells do not show proliferative activity. Therefore, it can be concluded that ongoing cell proliferation is not necessary for the upregulation of collagen-I gene expression in the same cell. Our data are consistent with several possible interrelations between cell proliferation and collagen synthesis. For example, many of the procollagen-positive cells could recently have been in the cell cycle within the past few days, given that an estimated half life of PCNA protein is only about 20 h [18]. We therefore cannot deny a possibility that cell proliferation plays a role in the initiation, rather than the maintenance of collagen synthesis, for example, via changes in so-called 'smooth muscle cell phenotype' [9, 10]. It is also possible that in vivo processes leading to collagen synthesis, or the recently deposited matrix, could have an inhibitory effect on cell proliferation.

Theoretically, certain stimulatory agents (growth substances, mechanical factors, etc.) could influence both growth-related processes [2–5]. If this were the case, one might expect to see a close spatial association between these two processes (i.e. PCNA-positive and procollagen-I-positive cells should both colocalize in the same regions of the plaque). Recently we performed two independent surveys on the morphological feature correlates of cell proliferation and collagen synthesis in human atherosclerotic plaques [11–13]. For the relatively rare cell proliferation, two features were highlighted: foam cell-rich regions and vascularized regions. For collagen synthesis, fibrous cap zones and vascularized regions were again highlighted. This is in concert with our present double-labeling data. The relatively small incidence of vascularized regions might help to explain the overall negative correlation seen in the current study.

Moreover, relationships between cell proliferation and collagen synthesis in human tissues with a 'salt and pepper' distribution of different cell types are far more complicated than in pure smooth muscle cell cultures. We previously demonstrated that the largest category of proliferating cells in human atherosclerotic plaque is represented by monocyte macrophages [11]. They, in turn, comprise almost the whole region around many necrotic cores and do not produce collagen. Thus the presence of proliferating monocyte macrophages and possibly some T cells partially explains why many of PCNA-positive cells would not be expected to display collagen gene expression. At the same time, the proliferative rate of smooth muscle cells (the major cell type in the fibrous cap region) in human atherosclerotic plaques is extremely low (0–1% of carotid plaque cells exhibiting PCNA immunoreactivity vs. about 15% of carotid plaque cells being positive for type-I procollagen protein [11–13]). This again suggests that cell proliferation and collagen gene expression are under separate controls in human atherosclerotic plaques.

Vascularized regions demonstrate both PCNA and procollagen positivity. So, at least in one definite part of the plaque, two main growth-related processes occur together. In human atherosclerosis, neovascularization of the intima is felt to be a common event, occurring relatively late in the development of the plaque, and often associated with complications such as thrombus or intraplaque hemorrhage organization [19–23]. Such microvessels are felt to come primarily from the adventitial vasa vasora, although occasional origin from the arterial lumen has been suggested. Recently, we also demonstrated that neovascularization plays an important role in the intimal growth in human arteriovenous fistulas, used for hemodialysis [24]. The PCNA index in microvessel-contained intimal fields was 5–6 times that of avascular fields. Double-label immunocytochemistry with anti-PCNA and cell type-specific antibody revealed that the vast majority of PCNA-positive cells was represented by microvascular endothelial cells and surrounding pericyte-like smooth muscle cells. This close spatial association may be due to

possible angiogenic factors such as acidic fibroblast growth factors in vascularized regions of human plaques [25] and to the possible production of smooth muscle growth factors by endothelial cells in such regions [26–33]. Studies by other investigators have also shown that plaque microvessel-rich areas are notable for a prominence of proliferative activity [34]. Thus, neovascularization seems to be one of the major mechanisms of atherosclerotic plaque growth both in terms of cell proliferation and collagen production. This might be in part because of growth factor production by actively growing endothelial and smooth muscle cells [35]. With respect to regional differences in patterns of collagen-I gene expression and cell proliferation, it is likely that different mechanisms of growth control are switched on and off in different regions of the atherosclerotic plaque, at different times.

To summarize, in human carotid plaques, proliferation and collagen synthesis can occur in the same cells, but the vast majority of collagen-producing cells do not show proliferative activity. These two growth processes, while they can coexist in certain regions of the plaque, are generally spatially separate. Thus although some growth factors can stimulate both processes among at least smooth muscle cells (e.g., platelet-derived growth factor, transforming growth factor-β), the spatial separation of these two processes in human atherosclerotic material suggests that they may be under separate growth stimulatory/inhibitory controls. An understanding of the growth factors involved in intimal proliferation and collagen synthesis may lead to effective methods for the treatment of atherosclerosis.

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References

1 Ross R: The pathogenesis of atheroslcerosis-an update. N Engl J Med 1986;314:488–500.
2 Rokosova B, Bentley JP: Effect of calcium on cell proliferation and extracellular matrix synthesis in arterial smooth muscle cells and dermal fibroblasts. Exp Mol Pathol 1986;44:307–317.
3 Munro JM, Cotran RS: The pathogenesis of atherosclerosis: Atherogenesis and inflammation. Lab Invest 1988;58:249–261.
4 Davidson JM, Zoia O, Liu JM: Modulation of transforming growth factor-beta 1 stimulated elastin and collagen production and proliferation in porcine vascular smooth muscle cells and skin fibroblasts by basic fibroblast growth factor, transforming growth factor-alpha, and insulin-like growth factor-I. J Cell Physiol 1993;155:149–156.
5 Orekhov AN, Tertov VV, Kudryashov SA, Smirnov VN: Triggerlike stimulation of cholesterol accumulation and DNA and extracellular matrix synthesis induced by atherogenic serum or low density lipoprotein in cultured cells. Circ Res 1990;66:311–320.
6 Stepp MA, Kindy MS, Franzblau C, Sonenshein GE: Complex regulation of collagen gene expression in cultured bovine aortic smooth muscle cells. J Biol Chem 1986;261:6542–6547.
7 Liau G, Chan LM: Regulation of extracellular matrix RNA levels in cultured smooth muscle cells. Relationship to cellular quiescence. J Biol Chem 1989;264:10315–10320.
8 Majors AK, Ehrhart LA: Cell density and proliferation modulate collagen synthesis and procollagen mRNA levels in arterial smooth muscle cells. Exp Cell Res 1992;200:168–174.
9 Okada Y, Katsuda S, Matsui Y, Watanabe H, Nakanishi I: Collagen synthesis by cultured arterial smooth muscle cells during spontaneous phenotypic modulation. Acta Pathol Jpn 1990;40:157–164.
10 Ang AH, Tachas G, Campbell JH, Bateman JF, Campbell GR: Collagen synthesis by cultured rabbit aortic smooth-muscle cells. Alteration with phenotype. Biochem J 1990;265:461–469.
11 Gordon D, Reidy MA, Benditt EP, Schwartz SM: Cell proliferation in human coronary arteries. Proc Natl Acad Sci USA 1990;87:4600–4604.
12 Gordon D, Reidy MA, Kollman G, Schwartz SM, Gown AM: Measures of cell proliferation in human coronary and carotid arteries using cyclin/PCNA immunocytochemistry (abstract). FASEB J 1989;3:1318.
13 Rekhter MD, Zhang K, Narayanan AS, Phan S, Schork MA, Gordon D: Type I collagen gene expression in human atherosclerosis. Localization to specific plaque regions. Am J Pathol 1993;143:1634–1648.
14 Botney MD, Kaiser LR, Cooper JD, Mecham RP, Parghi D, Roby J, Parks WC: Extracellular matrix protein gene expression in atherosclerotic hypertensive pulmonary arteries. Am J Pathol 1992;140:357–364.
15 Botney MD, Liptay MJ, Kaiser LR, Cooper JD, Parks WC, Mecham RP: Active collagen synthesis by pulmonary arteries in human primary pulmonary hypertension. Am J Pathol 1993;143:121–129.
16 Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, Senior RM, Deuel TF: Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanimsms. J Cell Biol 1989;109:429–440.
17 Foellmer HG, Kawahara K, Madri JA, Furthmayr H, Timpl R, Tuderman L: A monoclonal antibody specific for the amino terminal cleavage site of procollagen type I. Eur J Biochem 1983;134:183–189.
18 Bravo R, Macdonald-Bravo H: Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites. J Cell Biol 1987;105:1549–1554.
19 Barger AC, Beeuwkes R: Rupture of coronary vasa vasorum as a trigger of acute myocardial infarction. Am J Cardiol 1990;66:41G–43G.
20 Desinan L, Rocco M, Pizzolitto S, Floretti G, Antoci B: Parietal centripetal and centrifugal thickening neovascularization in the descending anterior coronary artery. Possible relations with the problem of collateral circulation. Virchows Arch A Pathol Anat Histol 1989;415:211–217.
21 Sueishi K, Yasunaga C, Castellanos E, Kumamoto M, Tanaka K: Sustained arterial injury and progression of atherosclerosis. Ann NY Acad Sci 1990;598:223–231.

22 Zamir M, Silver MD: Vasculature in the walls of human coronary arteries. Arch Pathol Lab Med 1985;109:659–662.
23 Cliff WJ, Heathcote CR, Moss NS, Reichenbach DD: The coronary arteries in cases of cardiac and noncardiac sudden death. Am J Pathol 1988;132:319–329.
24 Rekhter M, Nicholls S, Ferguson M, Gordon D: Cell proliferation in human arteriovenous fistulas used for hemodialysis. Arterioscler Thromb 1993;13:609–617.
25 Brogi E, Winkles JA, Underwood R, Clinton SK, Alberts GF, Libby P: Distinct patterns of expression of fibroblast growth factors and their receptors in human atheroma and nonatherosclerotic arteris. J Clin Invest 1993;92: 2408–2418.
26 Golden MA, Au YP, Kirkman TR, Wilcox JN, Raines EW, Ross R, Clowes AW: Platelet-derived growth factor activity and mRNA expression in healing vascular grafts in baboons. Association in vivo of platelet-derived growth factor mRNA and protein with cellular proliferation. J Clin Invest 1991;87:406–414.
27 Collins T, Pober JS, Gimbrone MA Jr, Hammacher A, Betsholtz C, Westermark B, Heldin CH: Cultured human endothelial cells express platelet-derived growth factor A chain. Am J Pathol 1987;126:7–12.
28 Koo EW, Gotlieb AI: Endothelial stimulation of intimal cell proliferation in a porcine aortic organ culture. Am J Pathol 1989;134:497–503.
29 Lonchampt MO, Pinelis S, Goulin J, Chabrier PE, Braquet P: Proliferation and Na+/H+ exchange activation by endothelin in vascular smooth muscle cells. Am J Hypertens 1991;4: 776–779.
30 Sitaras NM, Sariban E, Pantazis P, Zetter B, Antoniades HN: Human iliac artery endothelial cells express both genes encoding the chains of platelet-derived growth factor (PDGF) and synthesize PDGF-like mitogen. J Cell Physiol 1987;132:376–380.
31 Xu CB, Falke P, Stavenow L: Interactions between cultured bovine arterial smooth muscle cells and endothelial cells; studies on the release of growth inhibiting and growth stimulating factors. Artery 1990;17:297–310.
32 Borsum T: Biochemical properties of vascular endothelial cells. Virchows Arch B Cell Pathol 1991;60:279–286.
33 Wilcox JN, Smith KM, Williams LT, Schwartz SM, Gordon D: Platelet-derived growth factor mRNA detection in human atherosclerotic plaques by in situ hybridization. J Clin Invest 1988;82:1134–1143.
34 O'Brien ER, Alpers CE, Stewart DK, Ferguson M, Tran N, Gordon D, Benditt EP, Hinohara T, Simpson JB, Schwartz SM: Proliferation in primary and restenotic coronary atherectomy tissue. Implications for antiproliferative therapy. Circ Res 1993;73:223–231.
35 Eisenstein R: Angiogenesis in arteries: review. Pharmacol Ther 1991;49:1–19.